STUDIES ON THE BIOLOGY OF THE CHRYSOPIDAE V. THE DEVELOPMENTAL AND REPRODUCTIVE MATURATION RATES OF *CHRYSOPA CARNEA* NEUROPTERA: CHRYSOPIDAE) [1][2]

Joseph K. Sheldon,[3] Ellis G. MacLeod[4],[5]

ABSTRACT: The effect on the developmental rate of *Chrysopa carnea* Stephens of the variables photoperiod, temperature, geographic locality, diapause state, and sex was examined by multiple regression analysis. A significant three-way interaction between photoperiod, temperature, and locality was found. Both diapause state and sex were also found to have a significant effect on development, although neither were involved in interactions with other factors.

A study of the effect of photoperiod on the reproductive maturation rate of nondiapausing adults was also made. A significant difference was found in the response of females reared under LD = 14/10 vs. LD = 16/8. The significance appears to lie in the degree of heterogeneity of the female's response once yolk depostion has begun. No effect was found on the males examined.

Chrysopa carnea Stephens, one of the more common North American members of the ubiquitous family Chrysopidae, is widely distributed throughout the temperate regions of the world. Recently this species has gained attention as a potential biological control agent because of its highly predaceous larval stages (Lingren et al. 1968a,b; Ridgway and Jones 1969) and a number of studies examining aspects of its biology have appeared. These include work on the adult feeding habits (Hagen et al. 1970a, b; Sheldon and MacLeod 1971), characteristics of the diapause phenology (MacLeod 1967; Honek 1973; Honek and Hodek 1973; Tauber and Tauber 1969, 1970a,b, 1972, 1973a,b; Tauber et al. 1970a,b) , and more general studies of the over-all seasonal cycle (Zeleny 1965, Sheldon and MacLeod 1974).

The data presented here relate to yet another phase of the biology of *C. carnea*, the developmental and reproductive maturation rates. We have examined the influence of the variables photoperiod, temperature, geographic origin, diapause state, and sex, plus their interactions on the developmental rate from egg to adult. This is then followed by a consideration of the response of newly emerged, nondiapausing adults to different photoperiods above the diapause inducing level in order to determine whether, once the critical photoperiod has been surpassed, if there is a photoperiodic effect on the reproductive maturation rate.

MATERIALS AND METHODS

The investigation of the pre-imaginal developmental rate utilized the offspring of 54 field-collected adult females from 3 localities, Paducah, Kentucky (37°N, 16 females) ; Urbana, Illinois (40 N, 22 females) ; and Madison, Wisconsin (43°N, 16 females). The work was carried out over a period of about 2 years (1969-1970) and involved 5 rearing experiments which examined the effects of 18 different photoperiods ranging from LD = 10/14 to LD = 16/8 at 2 temperatures, 19°C and 25°C on the developmental rate of the 3 geographic stocks. Results of the five rearings were pooled for the final analysis. Approximately 2200 individuals were reared in the study. In each rearing, the adults utilized for oviposition had constant access to both food (described by MacLeod 1967) and water. They were maintained at 25°C, LD = 16/8 prior to and during egg collection and an equal number of eggs was collected daily from each female. These were randomly distributed among the photoperiod and temperature regimes utilized during the rearing. The larvae were maintained as described by MacLeod (1967) except for the addition of a relative humidity control (see Sheldon and MacLeod 1971). After the adults emerged, they were held for six days, dissected, and analyzed for diapause as described elsewhere (Sheldon and MacLeod 1974). The ultimate fate, diapause vs. nondiapause, was considered a variable in the analysis.

The offspring of 12 females collected at Urbana, Illinois on 9 and 12 June 1969 were utilized to examine the effect of photoperiod on the reproductive maturation rate. These females were maintained as a mass culture at 25°C, LD = 16/8. Eggs were collected daily and were placed individually into cotton-stoppered, 2-dram shell vials. They were then divided equally between the 2 photoperiodic regimes, LD = 14/10 and LD = 16/8. The larvae were reared as above. These 2 photoperiods were chosen because they are on opposite ends of the ecologically relevant, nondipause-inducing photoperiods experienced by the Urbana population. LD = 14/10 is near the

Table 1. ANOVA of time to reach successive growth stages of _Chrysopa carnea_. The error mean square is used as the denominator in the F ratio as test of significance. * < 5%, ** < 1%, *** < 0.5%, **** < 0.1%

| Source | df | MS values: Number of days to: | | | | | |
		1st Instar	2nd	3rd	Cocoon	Pupa	Adult
Regression	26	90.35****	276.73****	533.08****	841.83****	1378.20****	2606.40****
Temperature (T)	1	369.00****	1175.16****	2061.44****	3387.32****	5858.70****	11552.07****
Sex (S)	1	0.86*	9.12****	44.07****	129.36****	119.48****	139.62****
Locality (L)	2	0.41	5.66****	19.67****	19.03****	15.20****	26.11****
Photoperiod (P)	4	2.63****	15.98****	50.94****	88.65****	80.98****	110.86****
T x L	2	0.29	1.50	7.80***	3.69	4.31	7.00
T x P	3	5.83****	4.19****	4.60**	13.33****	15.51****	16.54****
L x P	6	0.21	1.67*	4.33****	9.31****	7.56****	5.42*
T x L x P	6	0.33*	0.55	0.74	4.74**	4.42*	3.32[a]
Diapause (cov.)	1	<0.01	2.37	9.53***	26.17****	26.66****	17.80**
Residual	2165	0.13	0.67	1.14	1.67	1.99	2.41
Terms dropped	34	0.12	0.80	1.16	1.17	1.55	2.37
Error	2131	0.14	0.67	1.14	1.68	2.00	2.41
R^2		0.889	0.832	0.849	0.858	0.893	0.929

[a] $T \times L^2 \times P^2$ and $T \times L^2 \times P^3$ terms significant at <5% level.

critical photoperiod, but enough above it that there is no diapause (Sheldon and MacLeod 1974). LD = 16/8, on the other hand, is approximately the longest photoperiod experienced at a latitude of 40°N.

Upon emergence, the adults of each photoperiod were separated by sex and were randomly divided into groups to be dissected at the adult ages shown in Figs. 4 and 5. Prior to dissection, the adults were maintained under their rearing conditions. The analysis of the degree of reproductive maturation in the females was based on a measurement of the diameter of the basal oocyte of the largest ovariole in each ovary. A diameter of approximately 0.41 mm indicates the presence of a mature oocyte, while a teneral or diapausing individual has ovarioles with a maximum diameter of about 0.07 mm. In the males the position of the sperm was noted. A shift of sperm from the testis to the seminal vesicle indicates a sexually mature individual.

With the exception of the males in the reproductive maturation experiment where a chi square test was utilized, the results of both studies were analyzed with a multiple regression analysis.

RESULTS

Developmental Rate. The results of our analysis are presented in Table 1. The analysis was partitioned such that the separate contributions of each instar from egg to adult could be determined. All possible interactions for main factors and the covariate were considered. Terms were dropped only after examination of their contributions to regression sum of squares indicated that the terms were unnecessary. Retained interactions involving locality include both its linear and quadratic components; retained interactions involving photoperiod include its linear, quadratic, and cubic components.

In the periods to the 1st instar, cocoon, pupa, and adult, the analysis indicates that T (temperature), L (locality), and P (photoperiod) are involved in three-way interaction in the sense that the effect of any one factor depends on the levels of the other 2 factors. It should be pointed out that for the adult stage, although the 3-way interaction was not significant, 2 of the terms, of the 6 that are included in this interaction, are significant at less that the 5% level. Therefore, there is significant 3-way interaction if the interaction is partitioned into its individual components; then the 2 terms would be shown as significant. In the case of the 2nd and 3rd instars, the overall 3-way interaction is not significant, nor is any of the 6 terms included in each of these interactions significant.

The high degree of significance that was found in the lower-level terms, especially in the 2nd and 3rd instars and the adult stage where the 3-way interaction was lacking or only partially present, should also be noted. Of

the 2-way interactions, L x P and T x P, all are significant with the excep-
tion of L x P in the 1st instar. Also T x L in the 3rd instar proved highly
significant. The main factors, T, L, and P were all significant with the ex-
ception of locality in the 1st instar. Sex (S) was also found to be highly
significant even though its interactions were not. The males consistently
developed faster than the females. Diapause, the covariate, was also found
to have a significant effect in all except the 1st 2 periods, the diapausing
individuals developing, on the average, slightly faster (0.46 days) than the
nondiapausers.

The developmental rates for all instars (sexes combined) of the
Paducah, Ky.; Urbana, Ill.; and Madison, Wisc. populations are shown
in Figs. 1-3 respectively. A close examination of these figures reveals where
some of the complexity lies. The effect of temperature alone is striking, but
it is also evident that the general shape of the photoperiodic response curve
is consistently different at the 2 temperatures (compare A and B in all 3
figures) , a feature which is not surprising considering the high degree of
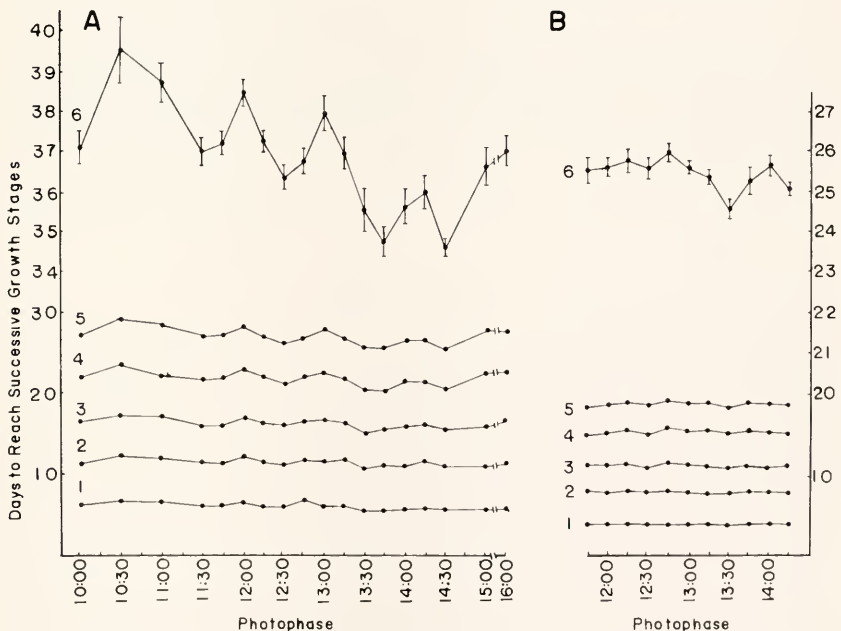significance for the T x P interaction shown in Table 1. The response of

Fig. 1. Time to reach successive growth stages of C. *carnea*, Paducah, Kentucky popula-
tion. A = $19°C$; B = $25°C$. 1 = the length of time to reach the 1st instar (hatching of egg) ; 2
= time to 2nd instar; 3 = time to 3rd instar; 4 = time to pre-pupa (spinning of cocoon; 5
= time to pupation; 6 = time to adult eclosion. The standard error for each photophase is
given for the adult.

Fig. 2. Time to reach successive growth stages of C. *carnea*, Urbana, Illinois population. A = 19°C; B = 25°C. 1 = the length of time to reach the 1st instar (hatching of egg); 2 = time to 2nd instar; 3 = time to 3rd instar; 4 = time to pre-pupa (spinning of cocoon); 5 = time to pupation; 6 = time to adult eclosion. The standard error for each photophase is given for the adult.
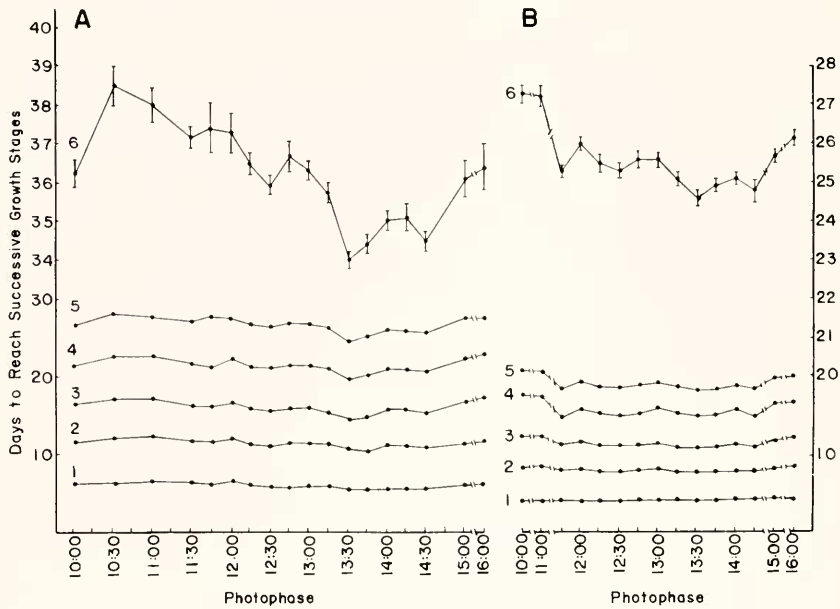

Fig. 3. Time to reach successive growth stages of C. *carnea*, Madison, Wisconsin population. A = 19°C; B = 25°C. 1 = the length of time to reach the 1st instar (hatching of egg); 2 = time to 2nd instar; 3 = time to 3rd instar; 4 = time to pre-pupa (spinning of cocoon); 5 = time to pupation; 6 = time to adult eclosion. The standard error for each photophase is given for the adult.
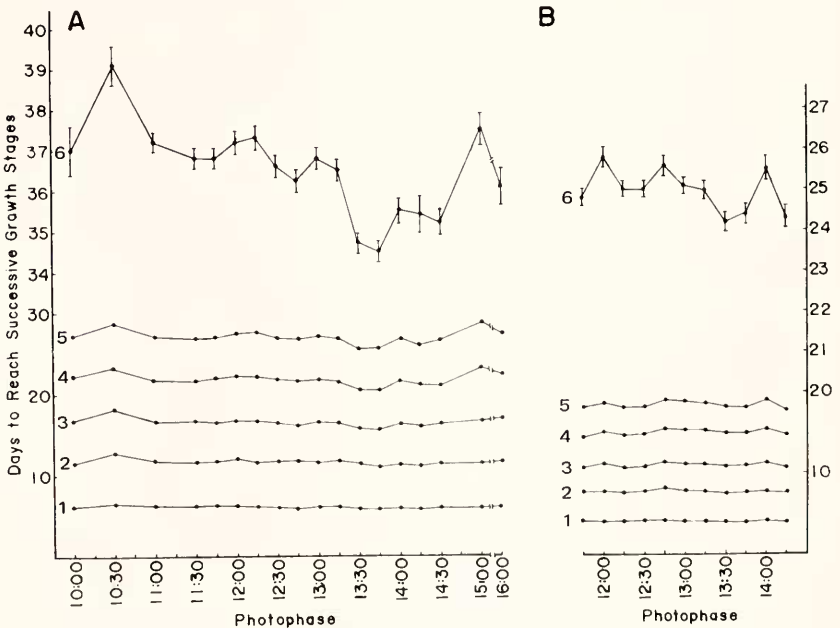
the 3 geographic populations also can be seen to vary with the temperature and photoperiod. A 19°C the Urbana population developed most rapidly under 12 of the 18 photoperiods examined, while Paducah was the slowest in 11 cases. The results were noticeably different at 25°C where the Madison population was found to develop most rapidly under 8 of the 11 photoperiods examined, with Paducah again the slowest 8 out of the 11 times.

Reproductive Maturation Rate. The results of this study are presented in Figs. 4 and 5. Females were found to initiate ovariole swelling, the first indication of yolk deposition, at about 48 h following adult eclosion and most individuals examined had mature eggs by 120 h (Fig. 4 A,B) . Some difference was present between the 2 groups with respect to the degree of variance once yolk deposition has begun. This was particularly apparent in the 84- and 108-h groups in the LD = 14/10 series in which there was significantly less yolk deposition present than in the corresponding groups at LD = 16/8. A differential response between the 2 groups was also indicated by multiple regression analysis (P <0.05) .

The results of the males (Fig. 5A,B) show that maturation occurs much earlier in this sex. The first males to undergo a sperm shift were encountered after 15 h in the LD = 14/10 group and after 21 h in the LD = 16/8 group. By 42 h all males examined at LD = 16/8 and all but 3 at LD = 14/10 had shifted sperm. The response of the LD = 14/10 group seemed to be somewhat more scattered (although not significantly so) , since the first shift appeared 6 h earlier and the last shift 6 h later than that found in the LD = 16/8 group.

DISCUSSION

Developmental Rate. That the developmental rate may be affected by a number of parameters has been common knowledge for many years. Most of the studies examining the influence of these parameters have been restricted to single factor analysis, with the effect of temperature receiving by far the most attention (see Wigglesworth 1965) . Other factors whose effect on the developmental rate have been examined include the length of the photoperiod (reviewed by Beck 1968, and dealt with in more recent studies such as those by Lutz 1968, Benschoter 1968, Clark 1969) ; sex (innumerable cases are known in which one sex develops faster than the other) ; and intraspecific differences due to geographic origin (Danilevskii 1965) .

Recently attempts have been made to examine simultaneously the influence of some of these environmental parameters to determine their joint effect (see for example Selander and Weddle 1972) . This latter approach has the potential of providing a great deal more information
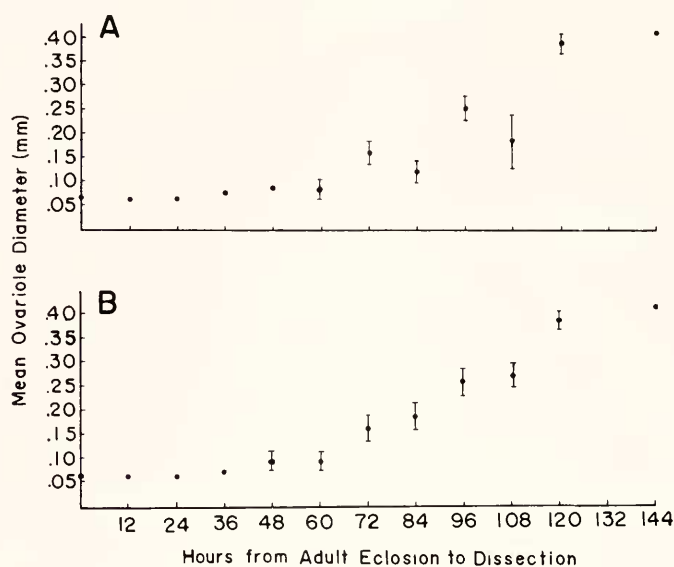
Fig. 4. The ovariole maturation rate of C. *carnea* females at two different photoperiod. A. LD = 14/10; B. LD = 16/8. N = 8 for each point. The standard error is given where equal to or greater than 0.01.
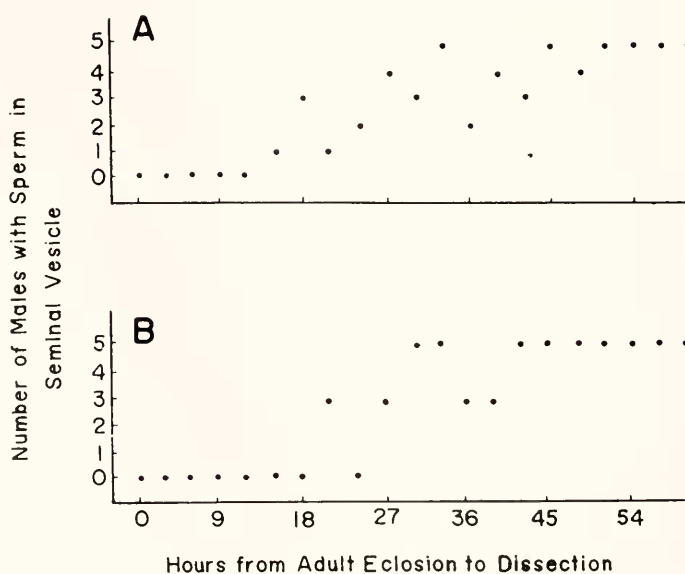

Fig. 5. The timing of the sequence of sperm shift from the testis to the seminal vesicle in C. *carnea* males at two different photoperiods. A. LD = 14/10; B. LD = 16/8. N = 5 for each point.

concerning the nature of the response as well as information about the organism's monitoring system since, as we have demonstrated, and it was also shown to be the case in the beetles examined by Selander and Weddle, multifactor control systems with high levels interaction are present in some species and are probably quite common. An attempt to deal with such complex problems by using single factor analysis will provide some information, but it is also likely to give a gross oversimplification of the actual conditions.

The significant 3-way interaction between temperature, locality, and photoperiod that we encountered in our analysis provides a good example of the complexity that may be involved. This is in marked contrast to the results reported by Benschoter 1968, working on *Heliothis zea* (Boddie) and *H. virescens* (Fabricius) who examined only the effect of photoperiod and found that an increase in the amount of light accelerated the developmental rate in approximately a linear manner. Although we found the slowest growth rates were at the short photoperiods, it was at the middle photoperiods that the most rapid development took place rather than at the long photoperiods as reported by Benschoter. This was true in all geographic populations at 19°C and in the Urbana group at 25° C. Unfortunately, the extreme photoperiods were not examined at 25°C for the Paducah and Madison strains.

The high degree of significance of the locality effect encountered in our study, outside of its part in the interaction, should also be noted since, in general, insects have not been found to show a geographic effect of locality on the developmental rate (Danilevskii 1965). Danilevskii suggests that this probably is due to the relatively uniform distribution of temperatures throughout the range of most species during the growing season. It would seem reasonable, however, that a southern population might develop more slowly at a given temperature e.g., Paducah, Ky. than its northern counterparts since the longer growing season would not necessitate a rapid growth rate to complete the life cycle-up to the point where additional generations are added. Since we have analyzed only the number of generations in the Urbana population (Sheldon and MacLeod 1974), we are unable to provide precise information on this point. The ecological reason why the Urbana population develops more rapidly at 25°C than Madison and then reverses itself at 19°C is also unclear at this time.

The more rapid development of the males would seem to indicate the presence of a selective advantage for a large early population of this sex. This same phenomenon was also encountered in the study of the overwintering generation discussed elsewhere (Sheldon and MacLeod 1974) in which the males were found to leave the overwintering sites first and undergo their sperm shift well before the females reach sexual maturity. Since both sexes undergo multiple matings (Sheldon and

MacLeod, unpublished data; Tauber and Tauber 1969) this would seem to maximize the likelihood of an adequate initial supply of males and, combined with the relatively long life span of both sexes (up to several weeks), it should also provide a sufficient number of males for subsequent matings.

Reproductive Maturation Rate. The initial motivation for this study was the need to determine the optimal time for performing dissections in the analysis for diapause in an investigation reported by Sheldon and MacLeod 1974. Individuals emerging in diapause cause no problems since their reproductive status remains constant, assuming that they are held under diapause maintaining conditions, for several weeks (see Sheldon and MacLeod 1974 and Tauber et al. 1970a for details). Our primary concern was the type of response shown by individuals reared at photoperiods very close to, but slightly above those inducing diapause vs. the typical long-day conditions during mid-summer.

It is clear, for the purpose of diapause analysis, that the response to the 2 photoperiods examined is essentially uniform. The difference in the rate of maturation encountered in the females was of little importance to the analysis since yolk deposition was initiated and mature eggs were encountered at approximately the same time in both groups. At $25°C$ a delay of 48 h in the dissection of males following adult eclosion provided sufficient time to permit those individuals not going into diapause to shift sperm to the seminal vesicle. Likewise, a delay of 5 days is sufficient for virtually all nondiapausing females to develop mature eggs.

The increased heterogeneity in the response at LD = 14/10 may indicate that at the shorter photoperiods, above the diapause-inducing levels, that individuals may not be entirely turned off to diapause. They may, in fact, undergo a periodic regression toward diapause which is then followed by another period of yolk deposition. It would seem likely that one might encounter some evidence of yolk resorption and/or an increase in the length of time required for development, however we did not detect either.

LITERATURE CITED

Beck, S.D. 1968. Insect photoperiodism. Academic Press, New York. 288p.

Benschoter, C.A. 1968. Diapause and development of *Heliothis zea* and *H. virescens* in controlled environments. Ann. Entomol. Soc. Am. 61:953-956.

Clark, S.H. 1969. Influence of photoperiod on development and larval diapause in the viceroy butterfly, *Limenitis archippus.* J. Insect Physiol. 15: 1951-1957.

Danilevskii, A.S. 1965. Photoperiodism and seasonal development of insects. Oliver & Boyd, Edinburgh and London. 283p.

Hagen, K.S., R.L. Tassan, and E.F. Sawall, Jr. 1970a. Some ecophysiological relationships between certain *Chrysopa,* honeydews and yeasts. Portici. Boll. Lab. Ent. agr, Filippo Silvestri. 28: 113-134.

Hagen, K.S., E.F. Sawall, Jr., and R.L. Tassan. 1970b. The use of food sprays to increase effectiveness of entomophagous insects. Proc. Tall Timbers Conf. Ecol. Anim. Control Habitat Manage. 2:59-81.

Honek, A. 1973. Relationship of colour changes and diapause in natural populations of *Chrysopa carnea* Steph. (Neuroptera, Chrysopidae). Acta ent. bohemoslov. 70: 254-258.

Honek, A., and I. Hodek. 1973. Diapause of *Chrysopa carnea* (Chrysopidae: Neuroptera) females in the field. Vestnik Cs. spol. zool. 37:95-100.

Lingren, P.D., R.L. Ridgway, and S.L. Jones. 1968a. Consumption by several arthropod predators of eggs and larvae of two *Heliothis* sup. (Lepidoptera: Noctuidae) that attack cotton. Ann. Entomol. Soc. Am. 61: 613-618.

Lingren, P.D., R.L. Ridgway, C.B. Cowan, Jr., J.W. Davis, and W.C. Watkins. 1968b. Biological control of the bollworm and the tobacco budworm by arthropod predators affected by insecticides. J. Econ. Entomol. 61:1521-1525.

Lutz, P.E. 1968. Effect of temperature and photoperiod on larval development in *Lestes eurinus*. Ecology 49:637-644.

MacLeod, E.G. 1967. Experimental induction and elimination of adult diapause and autumnal coloration in *Chrysopa carnea* (Neuroptera). J. Insect Physiol. 13:1343-1349.

Ridgway, R.L., and S.L. Jones. 1969. Inundative releases of *Chrysopa carnea* for control of *Heliothis* on cotton. J. Econ. Entomol. 62:177-180.

Selander, R.B., and R.C. Weddle. 1972. The ontogeny of blister beetles (Coleoptera: Meloidae). III. diapause termination in coarctate larvae of *Epicauta segmenta*. Ann. Entomol. Soc. Am. 65:1-17.

Sheldon, J.K., and E.G. MacLeod. 1971. Studies on the biology of the Chrysopidae II. The Feeding behavior of the adult of *Chrysopa carnea* (Neuroptera). Psyche 78:107-121.

Sheldon, J.K., and E.G. MacLeod. 1974. Studies on the biology of the Chrysopidae IV. A field and laboratory study of the seasonal cycle of *Chrysopa carnea* Stephens in Central Illinois (Neuroptera: Chrysopidae). Trans. Amer. Entomol. Soc. 100: 437-512.

Tauber, M.J., and C.A. Tauber. 1969. Diapause in *Chrysopa carnea* (Neuroptera: Chrysopidae) I. Effect of photoperiod on reproductively active adults. Can. Entomol. 101:364-370.

Tauber, M.J., and C.A. Tauber. 1970a. Photoperiodic induction and termination of diapause in an insect: response to changing day lengths. Science (Wash., D.C.) 167: 170.

Tauber, M.J., and C.A. Tauber. 1970b. Adult diapause in *Chrysopa carnea*: stages sensitive to photoperiodic induction. J. Insect Physiol. 16:2075-2080.

Tauber, M.J., and C.A. Tauber. 1972. Geographic variation in critical photoperiod and in diapause intensity of *Chrysopa carnea* (Neuroptera). J. Insect Physiol. 18:25-29.

Tauber, M.J., and C.A. Tauber. 1973a. Quantitative response to daylength during diapause in insects. Nature 244: 296-297.

Tauber, M.J., and C.A. Tauber. 1973b. Seasonal regulation of dormancy in *Chrysopa carnea* (Neuroptera). J. Insect Physiol. 19:1455-1463.

Tauber, M.J., C.A. Tauber, and C.J. Denys. 1970a. Diapause in *Chrysopa carnea* (Neuroptera: Chrysopidae) II. Maintenance by photoperiod. Can. Entomol. 102:474-478.

Tauber, M.J., C.A. Tauber, and C.J. Denys. 1970b. Adult diapause in *Chrysopa carnea*: photoperiodic control of duration and color. J. Insect Physiol. 16:949-955.

Wigglesworth, V.B. 1965. The principles of insect physiology. E.P. Dutton & Co. Inc., New York. 741p.

Zeleny, J. 1965. Lace-wings (Neuroptera) in cultural steppe and the population dynamics in the species *Chrysopa carnea* Steph. and *Chrysopa phyllochroma* Wesm. Acta ent. Bohemoslov. 62:177-194.